SENSORS AND PROBES

Tagging newborn proteins: version 2.0

Newly synthesized proteins of interest can be visualized with a tag by fluorescence imaging or electron microscopy.

Some years ago, Michael Lin, Roger Tsien and their colleagues at the University of California, San Diego, realized that none of the existing tools for tagging newly synthesized proteins of interest suited the needs of their experiment. So they decided to build their own. They developed TimeSTAMP, a genetically encoded tag that allowed them to visualize newly synthesized proteins of interest in a drug-controllable manner. The tagged protein was visualized by immunostaining in fixed samples. Now the authors have expanded TimeSTAMP to enable detection in live cells by fluorescence imaging or electron microscopy.

With TimeSTAMP, a cassette containing the protease of hepatitis C virus flanked by protease recognition sites on both sides is fused between the protein of interest and a tag. When expressed in cells, the tag is constitutively removed from the protein by the protease; but in the presence of a protease inhibitor drug, the protein retains its tag and can be visualized. To enable detection of the newly born protein by fluorescence, the group re-engineered the TimeSTAMP cassette by placing two fragments of a fluorescent protein at the two extremes. In the absence of protease activity during drug treatments, the two fragments come together to reconstitute a functional fluorescent protein. Without the drug, the tag is removed.

For electron microscopy, the group modified the fluorescent version of TimeSTAMP to incorporate the genetically encoded singlet oxygen generator miniSOG, which enables photo-oxidation for electron microscopy visualization. An alternative version of the cassette allows the tagged proteins to persist even after the drug is removed, which enables pulse-labeling experiments.

Lin, Tsien and their colleagues used the TimeSTAMP fluorescent tag to study new protein synthesis events related to activation of living cultured neurons. They tracked the synaptic protein PSD95 and found that local activity induces accumulation of this protein at synapses. They also used the photo-oxidizing tags to perform correlated light and electron microscopy in developing and mature neurons to define the precise locations at which PSD95 gets incorporated in these cells.

Erika Pastrana

RESEARCH PAPERS

Butko, M.T. *et al.* Fluorescent and photo-oxidizing TimeSTAMP tags track protein fates in light and electron microscopy. *Nat. Neurosci.* advance online publication (28 October 2012).